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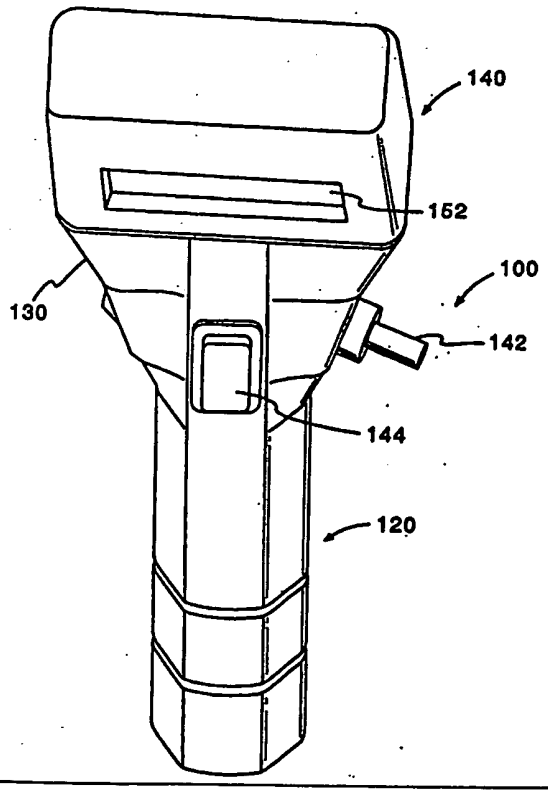
(54) Title: HAND-HELD LUMINOMETER

(57) Abstract

A hand-held luminometer (100) that serves as a self-contained assay device for measuring the presence of a target analyte by detecting and quantifying the light produced by a chemiluminescent reaction and displaying the results on a display (152). The luminometer has a handle portion (120) that is dimensioned so that the luminometer is easily gripped in an operator's hand.



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HAND-HELD LUMINOMETER

Field of the Invention

Embodiments of the present invention relate generally to a hand-held luminometer. One embodiment of the present invention relates to a hand-held luminometer that is adapted for performing chemiluminescent assays to detect target analytes, such as bacteria, on a contaminated surface.

Background of the Invention

The ability to rapidly and conveniently detect microorganisms is important for several industries. For example, the ability to detect bacterial contamination is paramount to improving food safety in food processing industries. During food processing, food can become contaminated with bacteria and spoil. Food poisoning can result if food contaminated with pathogenic bacteria, or its toxic products, is ingested without proper cooking.

Standard culture plate methods for monitoring surfaces for bacterial contamination require a sterile sample collection device (generally a swab or sponge) and suitable culture media, which after inoculation, must be incubated at a controlled temperature for a minimum of several hours to days. These methods are too cumbersome and time consuming, especially if used by untrained workers. Rapid bacteria tests need to be implemented in slaughterhouses and food handling establishments to improve safety. In these establishments, one must rapidly determine whether additional cleaning is required or whether proper safety procedures have been followed. To accomplish that, a quick, reliable bacteria

measurement is needed. Unfortunately, this is often not possible because present methods require several hours or even days and must be carried out by trained laboratory technicians, or require elaborate testing equipment that are not readily transportable to on-site locations.

Attempts have been made to overcome these problems by employing more sensitive chemiluminescence detection methods. One such chemiluminescence method measures adenosine triphosphate (ATP) to indirectly measure the bacteria content. This detection is reliable because all bacteria contain some ATP. Chemical bond energy from ATP is utilized in the chemiluminescent reaction occurring, for example, in the tails of the firefly *Photinus pyralis*. The mechanism of this chemiluminescence reaction has been well characterized (DeLuca, M., et al, 1979 Anal. Biochem. 95:194-198). The components of this reaction can be isolated free of ATP and subsequently used to detect ATP in other sources by a reaction that begins with formation of an enzyme bound luciferyl-adenylate complex and free inorganic pyrophosphate and ends with a rapid reaction of this complex with molecular oxygen to produce light, CO_2, and adenosine monophosphate (AMP).

One conventional light measuring method involves counting photons using a light reading instrument. Photographic films also have been used to monitor chemiluminescent reactions, as disclosed for example, in U.S. Patent 4,396,579. A drawback of this type is that it is complex and difficult to use.

Firefly luciferin-luciferase reactions have been used for detecting microorganisms, as described in U.S. Patents 4,385,113 and 5,366,867. These methods, however, suffer a number of deficiencies. Lyophilized luciferase-luciferin reagent is unstable at room temperature during long term storage and is unstable after liquid reconstitution over short time intervals. Additionally,

after reconstitution, the reagent solution emits significant amount of light even in the absence of ATP, which decreases detection sensitivity.

The reagent instability problem was partly addressed by drying luciferin-luciferase reagents separately onto plastic surfaces. But this requires an additional step of transferring microorganisms from a collection device to a plastic surface, increasing complexity. Further, while this solves the instability problem, it unfortunately lowers the detection sensitivity and creates a new problem — incomplete ATP transfer from the collection device to a separate plastic surface containing the luciferase-luciferin reagent. Furthermore, this solution introduces a new time variable between the transfer and the light emission measurement.

Adding reagent at timed intervals causes additional problems because the light emission kinetics become shorter as the light intensity decreases. The twin timing and reagent instability problems also plague other chemiluminescence chemistries that have been developed to detect target analytes. For example, U.S. Patent 4,396,579 describes a complex (and expensive), automated machine designed to add chemiluminescent reagent at fixed time intervals to overcome the light emission kinetic problem. The reagent instability and the timing problems make this machine unusually complex.

Thus, there is a need for an assay device that benefits from high sensitivity and speed of chemiluminescence detection, but excludes the aforementioned complexity, timing, reagent instability, and high background light emission problems.

Copending U.S. patent application SN. 08/560,094, filed November 17, 1995, entitled *CHEMILUMINESCENT ASSAY METHODS AND DEVICES FOR DETECTING TARGET ANALYTES*, describes chemiluminescent assay methods and devices that fulfill the afforementioned need. The disclosure of the

copending application is incorporated herein by reference. Further, copending U.S. patent application SN. 08/577,107, filed December 22, 1995, entitled *SAMPLING-ASSAY INTERFACE SYSTEM AND METHOD*, describes a sampling device that interfaces with the above-identified chemiluminescent assay methods and devices and thereby facilitates its use. The disclosure of this copending application is also incorporated herein by reference.

The above-mentioned copending applications disclose simple, easy-to-use chemiluminescent sampling assay devices and sampling device interfaces that eliminate or reduce the complexity associated with manually measuring and adding reagent at timed intervals. These sampling assay devices also provide means to measure light intensity and allow rapid analysis of target analytes at the sample site. One emodiment comprises a container or envelope holding a sampling strip having separate sampling and reagent portions. The reagent portion contains one or more dried chemiluminescent reagents. The device has a light-permeable portion that permits light generated by a chemiluminescent reaction within the sampling strip to exit the container. This sampling assay device eliminates or reduces much of the complexity associated with other known assay methods and, as a result, decreases the cost and training requirements for detecting target analytes. A wide range of target analytes can be detected by this device. In fact, the sampling portion of the device can collect virtually any type of target analyte, not only from physical contact with a solid, but also from sample liquid applied or introduced thereto.

In use, the sampling portion of the sampling assay device receives a sample, for example, by wiping the sampling portion over a surface suspected of containing the target analyte. A carrier liquid is then added to the sampling area, if needed, which liquid transports the

target analyte into the reagent portion. The carrier liquid also re-wets chemiluminescence reaction components located in the reagent portion, and thus allows a chemiluminescence reaction to begin. The instability problem suffered by prior art methods is overcome by providing the chemiluminescent reagent in a dried state within the reagent portion. A bacteriolytic agent (*e.g.*, a detergent) in the carrier liquid lyses bacteria that have been collected in the sampling portion. ATP liberated by lysis of bacteria then participates in a luciferase reaction to produce light. The advantage of rapid and sensitive detection of bacteria can be realized through sensitive light detection using, for example, a light detection device, such as, for example, a photomultiplier. The copending applications disclose, in essence, a compact, self-contained assay device that allows light detection using any of known light detection methods, including an optical observation.

Conventional instruments for measuring chemiluminescence, including luminometers and fluorometers, however, are not particularly suited for operation as a self contained assay device. In addition, most conventional luminometers are not designed to be hand-held in such a manner to permit a one-handed operation of the luminometer. Likewise, there are no hand-held luminometers that permit self contained assays that allow the insertion of the sample to form a sealed container within which the assay is performed and the results displayed.

Therefore, there is a need for a hand-held luminometer that can be used as a self contained sealed assay device. This device is needed so that a sample can be safely collected in a test site and then suitably inserted into the hand-held luminometer for detecting the target analyte and displaying the results at the site.

Since, these assays need to be performed at various different sites themselves, there is also a need for a device that is easy to carry and simple to operate. Preferably the device should be carried and operated using one-hand, so that efficiency of the test and the tester can be maximized.

Also, since the tests need to be performed at several different sites at several different times, there is a need for a device that minimizes the training and hiring costs of performing these tests. Thus, there is a need for a hand-held self contained assay device that requires fewer trained technicians to carry out the test and interpret the results on site so that corrective action can be initiated with minimum delay. Such rapid detection in turn triggers rapid corrective action that can bring significant savings by minimizing contamination, waste and spoilage at the test sites.

Summary of the Invention

Accordingly, one of the objects of the invention is providing a hand-held luminometer that can be adapted for use as a self contained assay device.

Another object of the invention is to provide a light-weight, portable, hand-held luminometer that can be held and operated in one hand by an operator requiring little training in order to perform rapid assays to detect target analytes at the monitored sites themselves.

These and other objectives are achieved by providing a hand-held luminometer having a handle portion, which is gripped in the hand, and a head portion attached to the hand portion. The head portion preferably is larger in size and lighter in weight than the hand portion for ease of use.

In a preferred embodiment the luminometer is held in one hand when a sample is applied. However, the

luminometer may be set onto a vertical or horizontal surface when not in use, or while the user waits for an assay result. To facilitate setting the luminometer onto a surface, the hand portion may be permanently or operably attached to a base portion. The base portion preferably comprises a cross sectional area which contacts a surface that exceeds the cross sectional surface of the hand portion. The base portion may comprise a power source to charge batteries in the luminometer when the luminometer is not being held.

The head portion may be attached horizontally or vertically to the handle portion. In one embodiment the head portion protrudes to the left of the handle portion so that when held with the left hand, the right hand can be used to insert a sample for analysis onto the handle of the device without interfering with the head portion. Other positions of the head portion with respect to the handle portion may be envisioned in accordance with the inventive principle of combining a convenient handle to hold the luminometer with a second identifiable portion that extends from the handle, or that alternately comprises a region of the handle that is not covered up with fingers during use.

In all cases the head portion comprises at least one of the electronic components of the luminometer, such as for example, a battery, computational device, light detector, light or aural signaling device. The head unit preferably comprises a readout device such as a display.

Either the handle portion or the head portion comprises a sample section which is adapted to receive a sample to be assayed. In a preferred embodiment the luminometer is shaped to have a head portion and a handle portion such that the height of the handle portion is at least fifty percent larger than the height of the head portion and the width of the handle portion is less than the width of the head portion. This shape of the

luminometer provides a handle that can be easily gripped by a user's hand and allows for one-handed carrying and operation of the luminometer.

In a preferred emobdiment, the handle portion of the luminometer includes a sample section including a light transmissive portion that interfaces with a sampling device having an assay portion for producing a chemiluminescent reaction with a target analyte. In another embodiment the sample section comprises a chamber.

In one embodiment, the handle portion also includes a measurement section with a light detection device that detects and quantifies light transmitted from the sample section as a result of the chemiluminescent reaction.

The head portion optionally may include a display/memory section with a data processing board that is connected to an output of the light detection device so that the quantity of detected light can be suitably stored in memory and displayed on the display device. The head portion also may include a joystick menu selection switch and a push button switch to facilitate a one-handed operation of the hand-held luminometer.

The sampling device also may include or be designed to work with, a cuvette that can be inserted into the sample section. The cuvette includes a sample portion having an enclosure that has an open first end, an open second end, and a tapered section such that the enclosure has a larger cross-section at the second end and tapers to a smaller cross section at the first end. Therefore, a standard sized swab can be inserted into the cuvette from the larger second end so that the swab is retained and squeezed by the smaller first end to extract any sample collected by the swab. The cuvette sampling device also can include a reading section contiguous to the smaller first end so that a test strip positioned

therein receives the sample extracted at the smaller first end.

Brief Description of the Drawings

Figures 1 through 8 which are incorporated in and constitute a part of the specification, illustrate presently preferred embodiments of the invention, and, together with the general description given above and the detailed description given below, serve to explain the principles of the invention. Figure 9 serves to explain prior art related to use of the invention.

Fig. 1 is a perspective view of a hand-held luminometer according to the present invention;

Fig. 2a is a left side elevation view of the luminometer;

Fig. 2b is a right side elevation view of the luminometer;

Fig. 3 is a schematic sectional view of the luminometer showing the internal structure of the luminometer;

Fig. 4 is a front elevation view of the luminometer;

Fig. 5 is a rear elevation view of the luminometer;

Figs. 6a and 6b are top and bottom plan views of the luminometer;

Figs. 7a, 7b, and 7c are front elevation, right elevation, and top plan views of a cuvette sampling device according to the invention;

Fig. 8 is a front/side perspective view of a flat sampling device according to the invention; and

Fig. 9 is schematic representation of a known photodetector connected to a known ammeter.

Detailed Description of the Preferred Embodiments

The present invention is directed to a chemiluminometer that can be held in one hand. In accordance with the objectives of the present invention, the chemiluminometer comprises a handle portion, which is gripped in the hand, and a head portion, which may contain electronics circuitry and which may contain a read-out device such as one or more light emitting diodes or a liquid crystal display. The head portion preferably contains a large sized display, such as a liquid crystal display for easy viewing by the user, and the shape of the handle portion is dictated by comfort to the user. In accordance with this design objective, the ratio between the handle portion and the head portion can be adjusted so that the dimensions of the handle portion is small enough so that the handle portion can be gripped easily in the hand.

In one preferred embodiment the handle portion is smaller than the head portion. For example, see Fig. 1, which is a perspective view of this embodiment of a hand-held luminometer 100 which includes a handle portion 120 that is contiguous with a head portion 140. As shown in the elevation and plan views of Figs. 2a, 2b and 4, the handle 120 has a handle length 120a, a handle width 120b and a handle height 120c. Likewise, the head portion 140 has a head length 140a, a head width 140b, and a head height 140c such that the handle height 120c is larger than the head height 140c and the handle width 120b is less than the head width 140b. In a preferred embodiment depicted in the figures, handle height 120c is at least twice the head height 140c and the handle width 120b is less than 50% of the head width 140b.

The actual sizes and ratio of the dimensions of the head portion 140 and the handle portion 120 is designed

so that the handle portion 120 can be easily gripped in the hand of a user or an operator. Therefore, the ratio can be varied so that the handle width 120b is approximately between 20% and 100% of the head width 140b and more preferably between 30% and 50% of the head width 140b. Handle height 120c can be approximately between one and four times the head height 140c and preferably between two and three times the head height 140c.

To facilitate the grip, the handle portion 120 optionally may be provided with external ridges 122 which ensure that the luminometer 100 can be securely gripped without slippage even when, for example, the user's hand is wet.

In a preferred embodiment, the head portion 140 is oblique to the handle portion 120 so that the luminometer 100 is optimally designed for hand-held use. The oblique arrangement enables easy reading of the luminometer by providing a display 152 on the head portion 140. A preferred angle between the head portion 140 and the handle portion 120 is within the range of from 10 degres to 90 degrees, for example, between 20 and 50 degrees, or between 30 and 40 degrees. The embodiment shown in Fig. 2 is approximately 34 degree. Of course, other angles that facilitate the use of the luminometer 100 can also be used.

The head portion 140 can include a transition portion 130 that fits the handle portion 120 so that there is gradual transition between the different dimensions of the head portion 140 and the handle portion 120. It should be understood that when the head portion 140 includes a transition portion 130 the dimensions of the head portion 140a, 140b, and 140c are measured at their maximum values.

It is to be understood that each of the handle portion 120 and the head portion 140, including the transition portion 130, can be manufactured as one

integral piece using conventional manufacturing techniques. Alternatively, the different portions can be suitably sealingly joined together using conventional fastening techniques, such as, for example, by heat, adhesive, ultrasonic welds, or any physical means that retain adjacent portions.

The head portion 140 of the luminometer 100 may include, for example, a joystick toggle switch 142 and a push button switch 144 that are positioned on the periphery of the head portion 140 so that they can be reached using the digits of one hand. For example, in the preferred embodiment, the joystick toggle switch 142 and the push button switch 144 can be positioned so that the thumb can operate the push button switch 144 while the forefinger can operate the joystick toggle switch 142.

This positioning of the push button switch 144 and the joystick switch 142 allows a one-handed operation of the luminometer because the handle portion can be gripped in the palm of one hand and the fingers of one hand can control the switches to operate the luminometer 100 using just one hand. It is to be understood that this relative positioning of the two buttons, 142 and 144, also permits either a left-handed or a right-handed operation of the luminometer 100.

In one embodiment, the joystick toggle switch 142 is designed as a multiple position toggle switch and in the preferred embodiment operates as a four-position toggle switch with the four positions corresponding to the 90, 180, 270, and 360 degree positions. These four positions can, for example, be used to select among four menu choices.

The push button switch 142 is a conventional switch that can be operated between two positions, such as, for example, an on and an off position.

The head portion 140 also includes a display/memory section 150 that has the display 152. In the preferred embodiment, the display 152 is a LCD display. However, any other display or read-out system can also be used as the display 152. Preferably, the display is protected by a non-glare surface or a non-glare screen can be fitted over the display to facilitate non-glare viewing of the display 152.

The handle portion 120 of the luminometer 100 includes a sample section 160 and a measurement section 170. The sample section 160 is hollow internally so that it is adapted to receive a sampling device such as the cuvette sampling device 200 that shown in Figs. 7a-7c and the described in the related text of this specification below.

The measurement section 170 includes a cavity that contains a light detection device 174 such as photo multiplier device, a charge coupled device ("CCD"), or a photon counting device.

The luminometer 100 is also includes a data processing board which is connected to an output from the light detection device 174. The data processing board 180 can process the output from the light detection device 174 and is connected to a display driver 154 which is connected to the display 150. Therefore, the luminometer 100 can be pre-programmed to have a variety of display formats and modes that display a wide range of data under the control of the operator.

The data processing board 180 can also be connected with a data transmission means 181 such as RS-232 connector or a wireless modem so that luminometer can communicate with a remote computer system. The luminometer 100 is also provided with a power supply that includes at least one battery.

Advantageously, one portion of the luminometer is adapted to receive a sample to be assayed. The portion

should provide a light-tight environment for measurement of the sample. In the embodiment shown, the sample section 160 is also provided with a hinged door 162 that is movable to a first position which opens a chamber associated with this section so that the sampling device 200 containing a sample to be assayed can be inserted into the sample section 160. The hinged door 162 can then be moved to a second position that closes and seals the sample section 160. The sample 160 has a light transmissive portion 164 can transmit any light generated in the sample section 160 to the measurement section 170.

Alternatively, the device could be adapted to receive the sample externally, provided that the sample-holder and device cooperate to create a light-tight environment in which to take a reading. For example, the sample could be placed into a sample holder that is releasably coupleable to the device. Upon coupling, a light-tight environment is created for measurement.

The measurement section 170 also may include a sapphire window 176 that aligns with light transmissive portion 164. The sapphire window 176 has a hardness that prevents scratching of the window from the insertion and removal of the sampling device 200 and also protects the light detection device contained in the measurement section 170.

Figs. 7a-7c show three views of an embodiment of a cuvette sampling device 200 that can be inserted in the luminometer 100. In this embodiment, the cuvette sampling device 200 is inserted into the sample section 160 through the hinged door 162 which can be opened for this purpose. Once the cuvette sampling device 200 is inserted into the sample section 160 the hinged door 162 is closed to provide a light proof seal. Thereafter, the chemiluminescent assay is suitably commenced so that the detected light can be quantified and displayed on the

display 152. The sealed sample section 160 also ensures that no sample or reagent escapes the luminometer 100.

As shown in the Figs 7a-7c, the cuvette sampling device 200 includes a sample portion 202 and an assay portion 206. The sample portion 202 includes an enclosure 204 that has longitudinally an open end first end 204a and an open second end 204b such that the open second end 204b has a larger cross-section than the open first end 204a. The enclosure 204 includes a tapered section 205 that leads to the smaller cross-section at the open first end 204a.

The cuvette sampling device 200 also includes a tab 207 that allows for convenient handling of the cuvette 200 so that it can be inserted and removed from the sample section 160 though the hinged door 162.

The first end 204a and the second end 204b are suitably dimensioned so that a standard swab can be inserted into the enclosure though the second end 204b having the larger cross-section but so that the swab is squeezed and retained by the tapered section 205 at the first end 204a having the smaller cross-section. Therefore, any sample collected by the swab is extracted by the squeezing action and flows through, by for example, a wicking action, onto the assay portion 206 of the cuvette sampling device 200.

The assay portion 206 is a flat enclosure that is contiguous with and extends outward from the open first end 204a and is suitable shaped with, for example, a shallow well, so that a test strip (not shown) can be positioned therein.

The test strip is composed of an adsorbent material, which may be fibrous, such as glass fiber, cotton, dacron, or paper and the like, and it may be porous, such as porous polyethylene or sintered glass and the like. An ordinary skilled artisan will recognize many useful

materials, such as those used in chromatographic-type assays currently available.

The assay portion 206, where the chemiluminescent reaction is designed to occur, contains one or more chemiluminescent reagents, preferably in a dried form. Additionally, the assay portion can contain other reagents useful for the assay including, for example, the detergent or other bacteriolytic reagent for extracting ATP from bacteria.

In another embodiment, a sampling device 300 is configured as a flat cassette, as shown in Fig. 8. This flat design advantageously allows alternative sample processing procedures to introduce a sample and reagent chemistry package into the instrument.

The sampling device 300 comprises a substantially flat, elongated, rectangular body 310 having a square hole 315 near one end and a removable flap 320 attached at the opposite end. The elongated body 310 has an elongated recess 335 that extends in the longitudinal direction, from the end where the flap is, toward the square hole 315. Formed through the recess is round hole 340. The recess ends before the square hole 315, but after the round hole 340. The flap 320 has an attachment ridge 330 that allows the flap 320 to fold or pivot relative to the body 310 to cover the round hole 340. In the closed position, the flap 320 nestles into the recess 335.

The body 310 also includes a tab 350 protruding at one of the sides, here at the right side from the user's perspective, to provide a foolproof way of inserting the sampling device 300. This tab 350 will only fit into the sample chamber in one orientation, with the square hole 315 leading into the machine.

For use in total ATP (adenosine triphosphate) detection or quantitation, at least one membrane 360, 370 may be positioned directly over the round hole 340, using

a clear tape affixed to the underside of body 310 to cover the hole 340. Luciferin and luciferase are dried onto the lower membrane 360 and a bacterial releasing agent, such as a detergent, may be dried onto the upper membrane 370. The lower surface of upper membrane 370 is made to contact the upper surface of lower membrane 360. Both membranes may be held in place by transparent tape affixed to the back side of hole 340 if the lower membrane is made slightly smaller (eg. about 0.5 mm diameter) than the upper membrane to expose an adhesive ring to retain the lower membrane (not shown).

A "Sontara" membrane, available from DuPont Chemical Company (Part Nos. 8818, 8801), comprising a blend of wood pulp and polyester, is particularly useful for these measurements because this type of membrane generally allows a low chemiluminescence background signal. In fact, the inventors surprisingly discovered that a membrane which is a blend of wood pulp with plastic polymer and, more particularly, blends of wood pulp with polyester are superior for chemiluminescence readout (light emission) reactions.

The Sontara membrane type preferred by the inventors comprises about 55% wood pulp and about 45% polyester, although variations in this ratio, (in particular between 10% wood pulp and 90% polyester to 90% wood pulp and 10% polyester) are possible and may be preferred for some applications. Of particular interest is a spunlaced fabric comprising staple fibers entangled through hydralic "needling" to form a strong, fabric-like structure. Non wood pulp fabric combinations, in contrast, yielded poorer signal to noise ratios for chemiluminescence. This advantageous property also is useful for other detection reactions, such as color development reactions that arise from enzyme catalysis of a chromogenic substrate.

Without wishing to be bound by a particular theory of the invention, the inventors point out that the inventive membrane has superior properties that allow water soluble reagents such as luciferin/luciferase and/or also hydrophilic drying agents such as sugars and polyols to dry down in the presence of cellulose, and allow interaction with the hydrophilic cellulose, while maintaining desirable properties such as strength and chemical inertness of the plastic polymer. In fact, an advantageous feature of the invention is to combine one or more biochemical reagents such as a protein with a drying agent into a cellulose pulp-plastic blend membrane. The membrane then is dried, preferably by lyophilization prior to storage and use in an assay.

In using the sampling device 300 for ATP detection or quantitation, a test sample is applied directly to upper membrane 370 either as a liquid drop or as a transfer of fluid from a moistened swab. The flap 320 is rotated or folded over so that it rests in the recess 335, thus covering the round hole 340 and the membranes 370 and 360. The sampling device is inserted into the luminometer, with the square hole end leading into the luminometer. After liquid from the sample enters both membranes and dissolves reagents found there, light is emitted through the round hole 340 and detected by the luminometer. The transparent tape (not shown) is affixed to the backside of hole 340.

The sampling device 300 advantageously can be used for more complicated sample processing that requires sequential reactions. One such type of sample processing involves lateral flow of fluid through two or more zones. For example, a strip of membrane material may be laid in the recess 335 and fluid applied near the attachment ridge 330. The applied fluid moves toward the round hole 340 by capillary action, during which the fluid may be filtered and/or reagent(s) present in the strip can mix

and participate in one or more chemical reactions. Chemiluminescence light generated from the reaction(s) is emitted and passed through the round hole 340, for detection by a light detector. The flap 320 itself can be transparent so that emitted light passes through flap 320 for detection by a light detector positioned above the hole 340.

As an example of more complicated sample processing for bacteria detection or quantitation, particulate matter such as that found in food samples can be removed and labeled enzyme can be attached to the bacteria before entering the final test zone over the hole 340. In one preferred embodiment, a membrane in the vicinity of the hole 340 contains dried enzyme substrate, or the substrate is added in a second step to the membrane before placing the flap 320 into the recess 335 and inserting the strip into the luminometer for detection of light.

Another example of more complicated sample processing is to use absorbent material attached to the flap 320 to control fluid that is applied to the membrane. In this case, a membrane can be supported in the hole 340 using an annular shaped adhesive tape such that the absorbent repeatedly contacts the membrane by closing the flap 320. In this embodiment various samples and reaction reagents can be added to the membrane and allowed to remain in the membrane for a longer time compared to that possible with lateral flow, as described above. As a result, longer reaction times are possible. The absorbent material may be brought into contact with the pad and excess reagents washed through or absorbed from the pad before addition of a subsequent reagent. After necessary sampling step(s) are completed, including the step(s) of adding luminescent substrate(s) if needed, the flap may be closed and the cassette inserted into the luminometer for determination of a light signal.

A wide variety of chemiluminescent chemistries can be used with the present hand-held assay device. Acceptable chemiluminescence chemistries include, among others, the reaction of hydrogen peroxide with horseradish peroxidase labelled antibodies and luminol, enhanced horseradish peroxidase, reactions that include the use of diacylhydrazides, acridinium salts, dioxitanes, and bioluminescent reactions involving cofactors, such as reduced nicotine adenine dinucleotide in the case of marine bacteria. A particularly preferred chemiluminescent chemistry is the firefly ATP assay, which utilizes luciferase and at least one cofactor to generate light from ATP.

At least one chemiluminescent reaction reagent is present in the assay portion 206, preferably in a dry state. When preparing the assay portion, reagents may be conveniently applied as a solution and then dried or they may be applied in a dry form, such as a powder or suspension in an organic solvent or slurry. Other methods are known in the art and the preferred one can be determined by characteristics of the reaction components desired.

Additionally, carrier liquid stored in a reservoir (not shown) can be used, as described in the aforementioned copending applications.

The carrier liquid preferably includes a bacteriolytic agent that releases ATP from any bacteria present in the sampling portion. Acceptable carrier liquids include, among others, a buffer solution or a buffer solution with detergent. Buffer solutions of TRIS, HEPES buffers at pH 7.0 to 9.0, and most preferably HEPES buffer at 7.8 with EDTA are preferred when used with firefly luciferase from *Photinus pyralis*. EDTA is a preferred ingredient because ATP degrading enzymes require divalent metal cations for activity and EDTA chelates these. Detergent, which can also be present in

the sampling or assay portion or included with the carrier, dissolves in liquid added to the sampling device and serves to open cells and liberate cell components. Several suitable detergents or combination of detergents are known to those skilled in the art and include, nonionic detergents such as Triton X-100, Nonidet P40, n-Undecyl Beta-D glucopyranoside, Zwitterionic detergents such as n-hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, and cationic detergents such as alkyltrimethylammonium bromides, benzalkonium chloride, cetyldimethyl-ethylammonium bromide, dodecyltrimethyl-ammonium bromide, and cetyltrimethylammonium bromide. The concentration of detergent solution varies for each type of detergent and can range from 0.1% to 6%, and preferably from 0.5% to 2.0%.

Devices for detecting intensity of chemiluminescent light are generally known. Chemiluminescent light, for instance, can be detected electronically by, for example, a photomultiplier, photo diode, photofet or charge coupled device. The most preferred is a photomultiplier because of its sensitivity. One known photomultiplier is disclosed in HAMAMATSU PHOTONICS K.K., Photosensor Modules H5773/H5783/H5784 Series, Technical Data, January 1995, the disclosure of which is incorporated herein by reference. The photomultiplier can be, for instance, Module H5773, a schematic functional diagram of which is illustrated in Fig. 8.

Referring to Fig. 8, the photomultiplier 50, such as the one disclosed in the aforementioned HAMAMATSU publication, has a housing 52 encasing a phototube 54 and an associated circuitry 56 for outputing a signal correlating to the light intensity detected. The photomultiplier 50, which can be positioned in the measurement section 170, can be connected to a conventional light intensity quantifier 57, such as a KEITHLEY Model 485 Picoammeter (ammeter), which measures

low current in the range between 100 Fa to 2 Ma. The phototube is accessed through the opening 58 formed in the housing 52.

The light intensity emitted from the chemiluminescent reaction obeys an inverse square relationship to distance following Lambert's Law. Therefore, if a light detector is used, detection sensitivity is optimized by placing the detector as closely as possible to the assay portion 206. The firefly luciferase from *Photinus pyralis* is 62,000 daltons and catalytically active in its monomeric form. The firefly luciferase reaction has a quantum yield of 0.88, which is the highest efficiency for any known bioluminescent reaction. At the optimum reaction pH of 7.8, the light emission is 562 nm. As the pH shifts to acidic conditions, the light emission has a second peak at 616 nm, which increases in intensity while the 562 nm peak decreases. The pH of the reaction determines the ratio of the 562 nm peak to the 616 nm peak. At pH 5.4, the 616 nm peak is at a maximum and there is no 562 nm peak. The emission spectra change with pH due to the protonation of the oxyluciferin molecule (the light emitter). Oxyluciferin as a dianion emits a yellow-green light (562 nm) and as a monoanion it emits a red light (616 nm).

Photomultiplier, such as the HAMAMATSU Module H5773/H5783/H5784 Series photosensors, have a highest spectral response between 400 and 500 nm, with decreasing responses above and below. Thus, the spectral response is greater at 562 nm than at 616 nm. In this regard, it would be desirable to run the luciferase assay at 7.8 pH to ensure a higher efficiency at the 562 nm peak emission. A filter can also be provided to block out a certain range of spectra to enhance detection.

Given the disclosure of the present invention, one versed in the art would readily appreciate that there may

be other embodiments and modifications well within the scope and spirit of the present invention. Accordingly, all expedient modifications readily attainable by one versed in the art from the present disclosure within the scope and spirit of the present invention are to be included as further embodiments of the present invention.

Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification be considered as exemplary only, with the true scope and spirit of the invention being indicated by the following claims.

What Is Claimed Is:

1. A hand-held luminometer comprising:

(a) a handle portion, and

(b) a head portion attached to said handle portion,

wherein said luminometer further comprises a sample section which is adapted to receive a sample to be assayed.

2. A hand-held luminometer comprising:

(a) a head portion having a head length, a head width, and a head height; and

(b) a handle portion, contiguous with said head portion, said handle portion having a handle length, a handle width, and a handle height,

wherein said handle height is at least 50% larger than said head height, and

wherein said handle width is equal to or less than said head width so that said handle portion can be gripped in an user's hand.

3. A hand-held luminometer according to claim 1, wherein said head portion is oblique to said handle portion and said handle width is less than 50% of said head width.

4. A hand-held luminometer according to claim 1, wherein said handle width is approximately between 30% and 50% of said head width.

5. A hand-held luminometer according to claim 1, wherein said handle height is approximately between 2 and 3 times said head height.

6. A hand-held luminometer according to claim 1, wherein the head portion includes a display/memory section and a joystick switch that allow a one-handed operation of the hand-held luminometer.

7. A hand-held luminometer according to claim 6,

wherein said joystick switch operates as a multiple position toggle switch, and

wherein said head portion further includes a push button switch.

8. A hand-held luminometer according to claim 7, wherein said joystick switch operates as a four position toggle switch.

9. A hand-held luminometer according to claim 1, wherein said handle portion includes a sample section and a measurement section.

10. A hand-held luminometer according to claim 6, wherein said display/memory section includes a display having a display length and a display width,

wherein said display width is approximately between 50% and 90% of said head width and said display height is approximately between 30% and 60% of said head height, and

wherein said display is protected by a non-glare surface.

11. A hand-held luminometer according to claim 10 wherein said display is an LCD display.

12. A hand-held luminometer according to claim 9, wherein said sample section is adapted to receive a sampling device, said sampling device including

a sample receiving portion, and

an assay portion connected to said sample receiving portion, said assay portion being suitable for carrying out a chemiluminescent reaction

wherein said sampling device comprises a light transmissive portion that is capable of transmitting light generated in said assay portion.

13. A hand-held luminometer according to claim 12, wherein the measurement section includes a cavity containing a light detection device, wherein said cavity

is proximate said light transmissive portion so that light produced by a chemiluminescent reaction in said assay portion is transmitted to and detected by said light detection device.

14. A hand-held luminometer according to claim 13, wherein said light detection device includes one of a photomultiplier device, a CCD, or a photon counting device.

15. A hand-held luminometer according to claim 1, wherein said handle portion further includes a data output means.

16. A hand-held luminometer according to claim 15, wherein said data output means includes one of a RS-232 connector socket and a wireless modem.

17. A hand-held luminometer according to claim 13, wherein an output of said light detection device is connected to a data processing board.

18. A hand-held luminometer according to claim 17, wherein said data processing board is connected to a display driver that controls a display device.

19. A hand-held luminometer according to claim 1, further comprising a power supply.

20. A hand-held luminometer according to claim 19, wherein said power supply includes at least one battery.

21. A hand-held luminometer according to claim 20, wherein said power supply further includes a power supply board.

22. A hand-held luminometer according to claim 1, wherein said handle portion has external ridges to permit non-slip gripping of said hand-held luminometer by an user's hand.

23. A hand-held luminometer according to claim 7, wherein said joystick switch and said push button switch are positioned so that said hand-held luminometer may be operated by either a left-handed or a right-handed operator.

24. A hand-held luminometer according to claim 9, wherein said sample section includes a hinged door that is movable to a first position that opens said sample section for insertion of a sampling device into said sample section, and

wherein said hinged door is movable to a second position that seals said sample section.

25. A hand-held luminometer according to claim 13, wherein said measurement section includes a sapphire window that aligns with said light transmissive portion,

wherein said sapphire window has a hardness that both protects said light detection device and prevents scratching of said sapphire window.

26. A hand-held luminometer according to claim 12, wherein said sampling device includes a cuvette, said cuvette comprising:

a sample portion including

an enclosure having longitudinally an open first end and an open second end,

wherein said enclosure has a larger cross-section at said open second end and includes a tapered section leading to a smaller cross-section at said open first end, and

wherein said first end and said second end are dimensioned such that a swab can be inserted into said enclosure through said second end and said swab is retained and squeezed by said tapered section and said first end to extract a sample collected by said swab; and

a reading section contiguous to said open first end such that a test strip positioned therein receives the sample extracted at said first end.

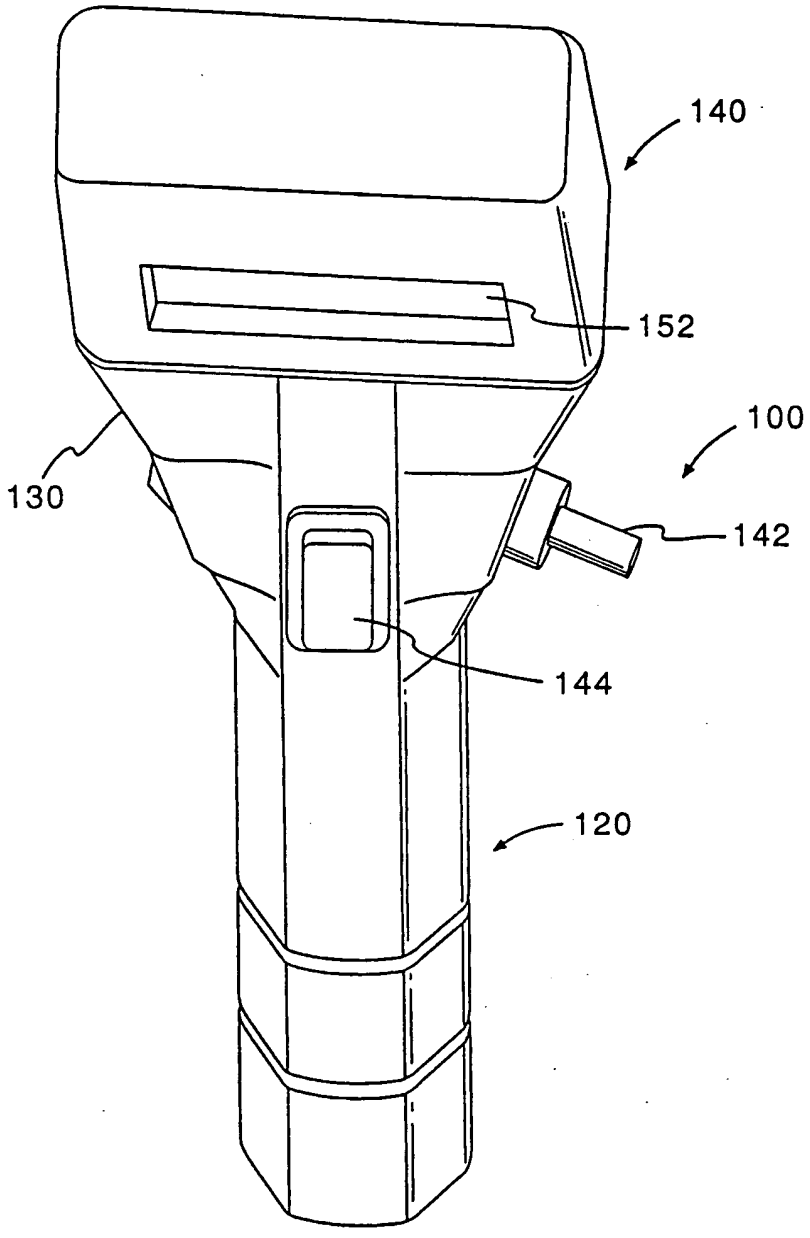

FIG. 1

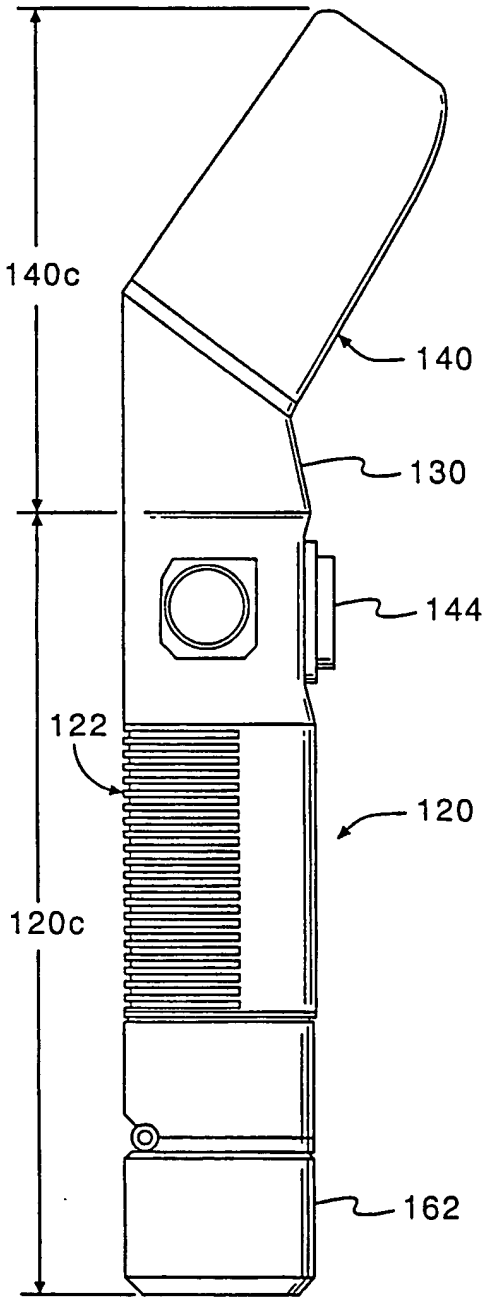

FIG. 2a

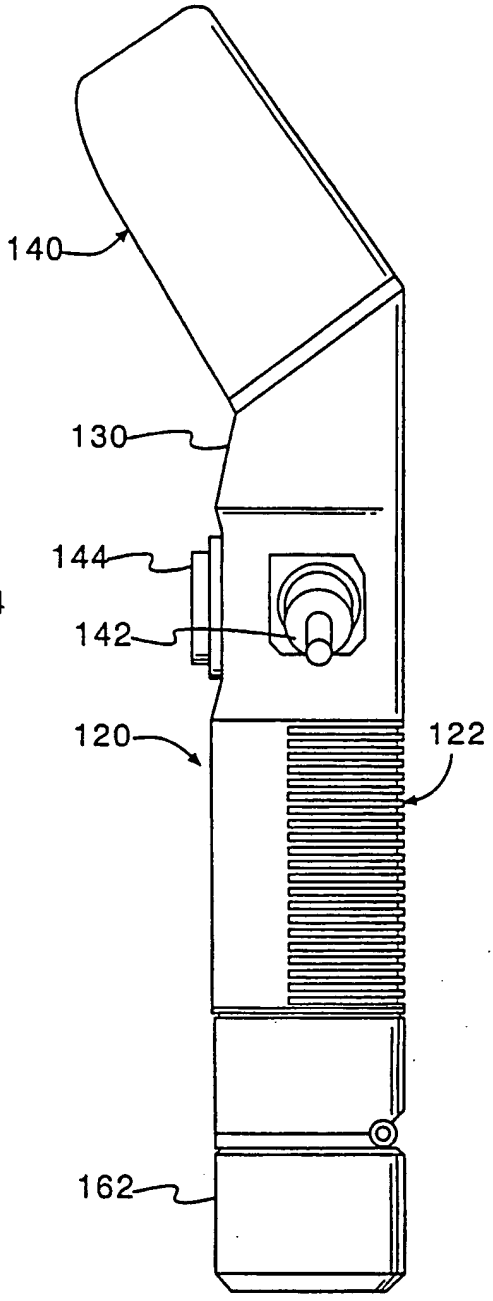

FIG. 2b

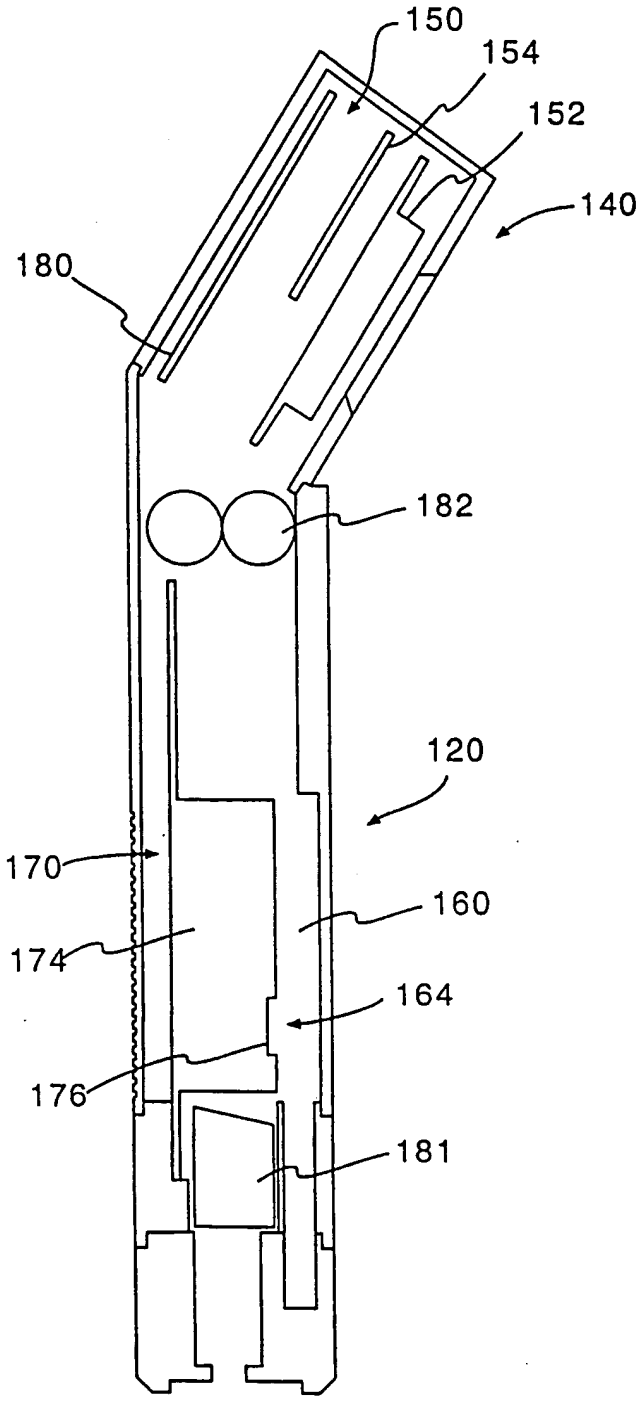

FIG. 3

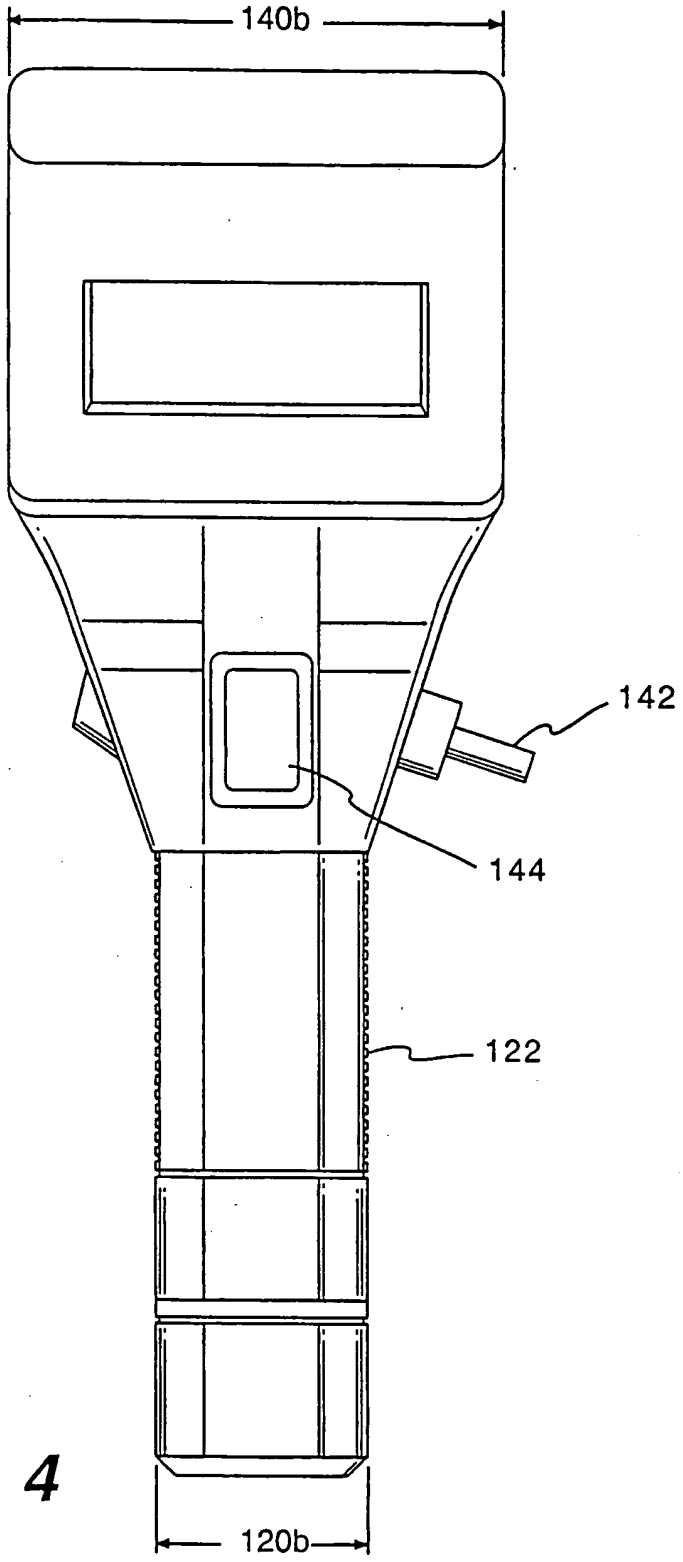

FIG. 4

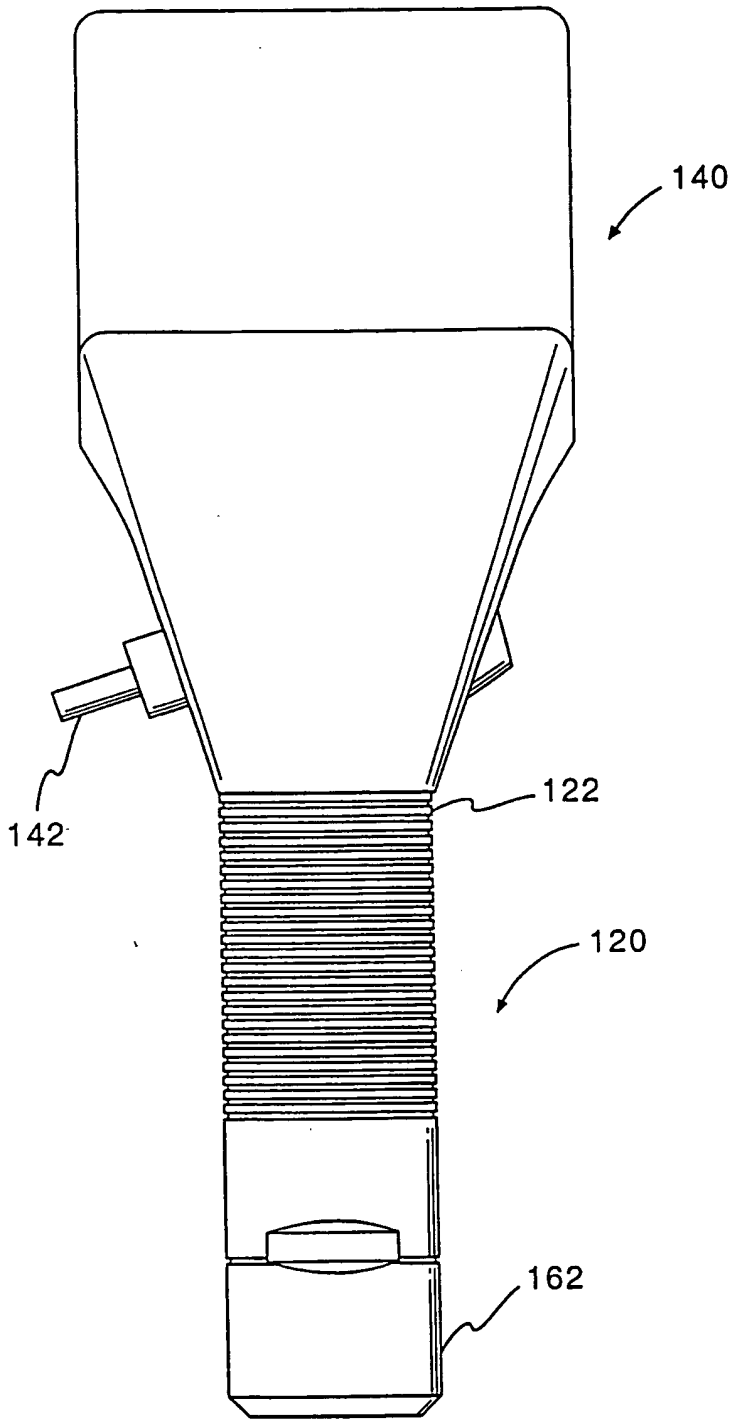

FIG. 5

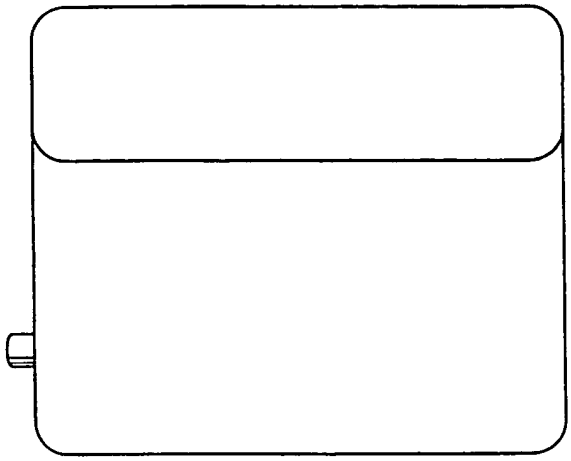

FIG. 6a

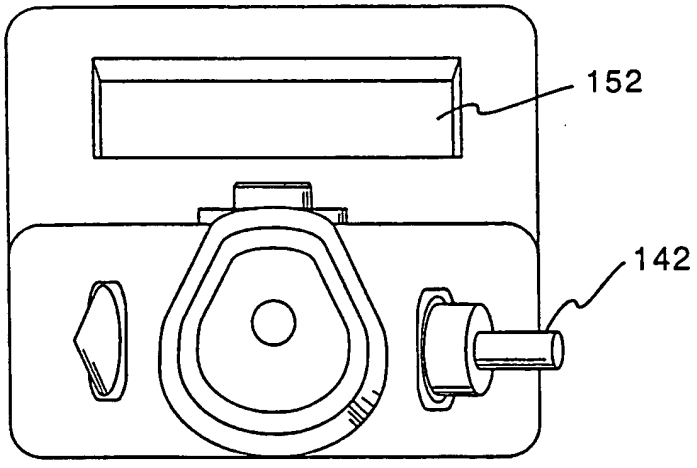

FIG. 6b

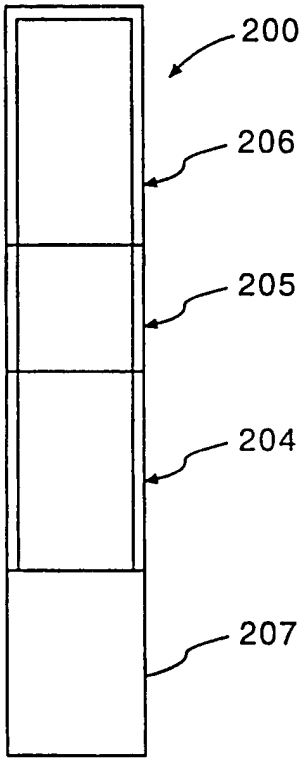

FIG. 7A

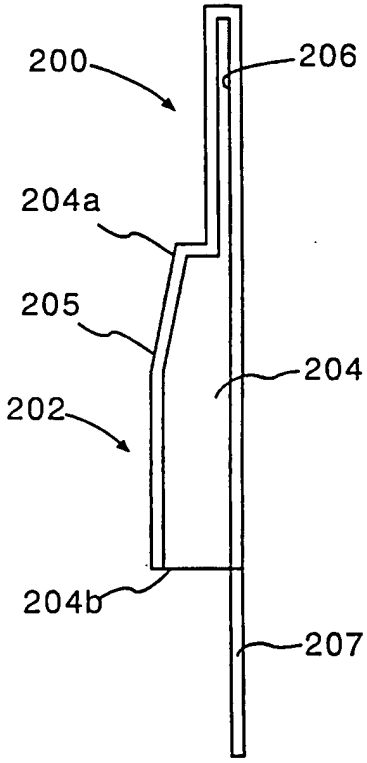

FIG. 7B

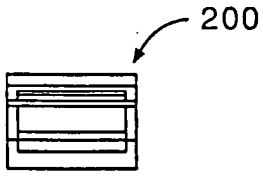

FIG. 7C

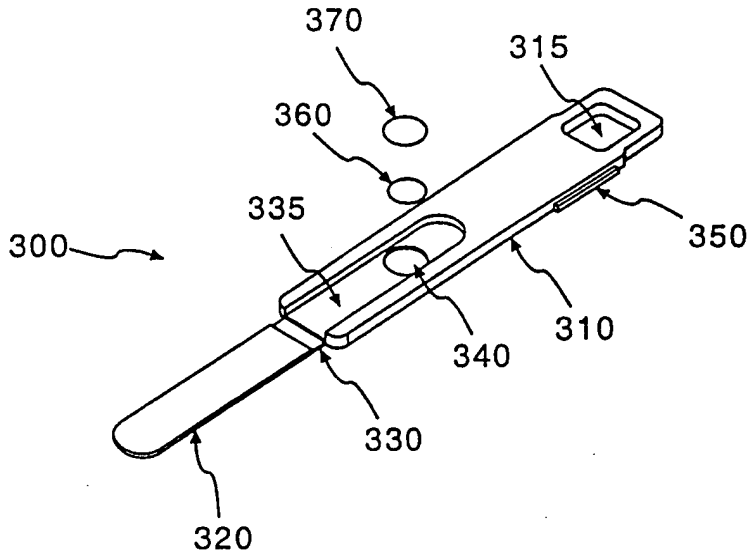

FIG. 8

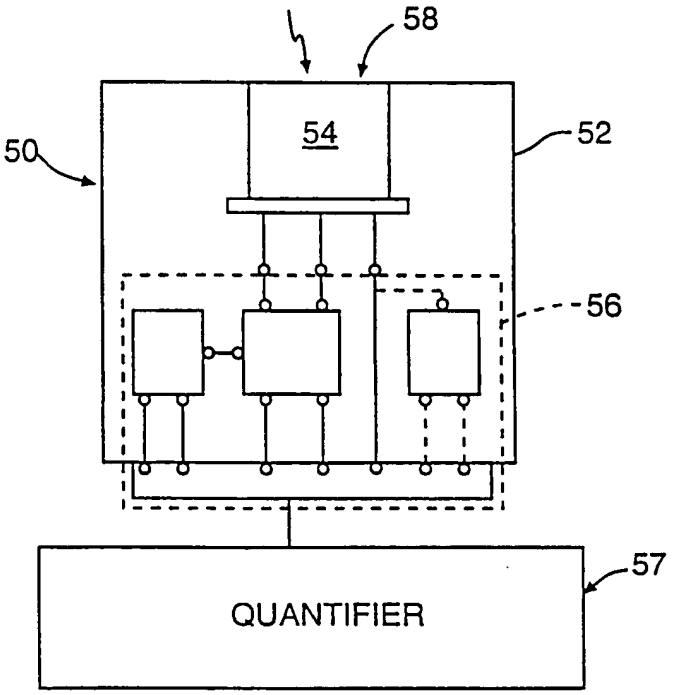

FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/08551

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :G01N 21/76
US CL :422/82.08; 250/361C, 458.1, 461.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/82.08; 250/361C, 458.1, 461.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,580,785 A (STIFFEY et al) 03 December 1996, entire document.	1-26
Y	US 5,576,550 A (KOPPIKAR) 19 November 1996, entire document.	1-26
Y	EP 0,357,625 B1 (BUNCE et al) 14 March 1990, entire document.	1-26
A	US 5,474910 A (ALFANO) 12 December 1995, Figure 3.	1-26
A	US 4,117,338 A (ADRION et al) 26 September 1978, entire document.	1-26

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
08 JUNE 1998

Date of mailing of the international search report
08 July 1998

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Form PCT/ISA/210 (second sheet)(July 1992)★